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Brief Communication: Resistance to *Falciparum* Malaria in α-Thalassemia, Oxidative Stress, and Hemoglobin Oxidation

GIOVANNI DESTRO-BISOL,[1]* ERNESTO D'ALOJA,[2] GABRIELLA SPEDINI,[1] ROBERTO SCATENA,[3] BRUNO GIARDINA,[3] AND VINCENZO PASCALI[4]
[1]*Department of Animal and Human Biology, University of Rome "La Sapienza," 00185 Rome, Italy*
[2]*Immunohematology Laboratory, Institute of Legal Medicine, Catholic University, 00168 Rome, Italy*
[3]*Institute of Chemistry and Clinical Chemistry, Catholic University, 00168 Rome, Italy*
[4]*Forensic Biology Department, University of Verona, 37100 Verona, Italy*

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ABSTRACT A recent survey conducted on Vanuatu Island suggests that resistance to *Plasmodium falciparum* in α-thalassemic individuals may have an immunological basis. This study is important since it seems to undermine the current idea that red-cell genetic defects give protection against *falciparum* malaria by reducing intraerythrocytic growth and development of the parasite. However, the mechanisms underlying these clinical and genetic observations are not yet fully understood. Based on a review of the relevant literature, we first show that the model based on the interaction between hemoglobin (Hb) and membrane components may provide a molecular basis for the involvement of the immune response in genetic adaptation to malaria. Second, we discuss the main evolutionary implications of the model. Finally, we suggest two approaches by which anthropological studies could provide a useful way of testing the model: 1) analysis of the interactions of malaria-resistance genes with genetic polymorphisms which affect the erythrocyte redox status and 2) study of the antimalarial effects of natural products (introduced as a part of a diet or for traditional antimalarial therapy) capable of interfering with the Hb/membrane interaction. Am J Phys Anthropol 109:269–273, 1999.

Genetic adaptation to malaria is commonly regarded as the most important example of how natural selection could drive the evolution of the human genome (Livingstone, 1971; Motulsky, 1989). Physical anthropologists are particularly interested in developing a biocultural perspective of population adaptation to malaria, the importance of which is shown by some important insights gained on the interactions between genetic, environmental, and cultural factors (e.g., Jackson, 1991; Etkin, 1997; Greene, 1997; Brown, 1997). However, a substantial increase in information on the molecular basis underlying genetic resistance to the disease is of primary importance to better understand how human populations develop protection from malaria.

We previously proposed a model which posits the molecular basis of some malaria-resistance genes in the interaction between

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*Correspondence to: Giovanni Destro-Bisol, Department of Animal and Human Biology, University of Rome "La Sapienza," P.le A. Moro 5, 00185 Roma, Italia.
E-mail destrobisol@axrma.uniroma1.it

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oxidized hemoglobin (Hb) and membrane components (Destro-Bisol et al., 1996). The model is supported by substantial evidence which indicates that erythrocytes of genetically protected individuals (carriers of the sickle cell trait, α- and β-thalassemia, and G6PD deficiency) are susceptible to the increase of oxidation of hemoglobin following H_2O_2 release in the host cell by *Plasmodium falciparum* (*P. falciparum*) and pyrexia which occurs during the periodic rupture of erythrocytes by the parasite. In their transition towards higher oxidation states (methemoglobin, reversible hemichromes, irreversible hemichromes and Heinz bodies), hemoglobin and its derivatives begin to cross-link or bind irreversibly with band 3 and spectrin, with a noteworthy effect also on lipids. This irreversible pattern of hemoglobin-membrane interaction could trigger mechanisms that 1) reduce invasion of erythrocytes by the falciparum parasite, 2) impair parasite survival and development within the cell, and 3) accelerate infected erythrocyte clearance by phagocytosis.

A new important insight into the molecular mechanism of malaria adaptation has recently come from a study on a cohort of thalassemic individuals on Vanuatu Island (Republic of Vanuatu) in the Pacific. This study showed that α^+-thal homozygous (-α/-α) children (less than 5 years old) contract precocious (if mild) malaria and splenomegaly, but they develop better immunity to the disease in the adult years. The crucial role the authors attribute to the immune system is in contrast with the established theory that resistance to malaria due to red-cell genetic defects is mainly due to reduced intraerythrocytic growth and the development of the parasite (Nagel, 1990). However, Williams et al. (1996) do not address the basic point concerning the molecular mechanisms which underlie the occurrence of malaria in α^+-thalassemic children.

In this study, we show how the interaction between oxidized Hb and the red-cell membrane may provide a plausible molecular basis for both the increased susceptibility to malaria in childhood and the improved immune response against *P. falciparum* in α^+-thalassemic children. Then we discuss some of the more important evolutionary implications of the model, and we suggest how it could be tested in anthropological studies.

THE HB/MEMBRANE INTERACTION MODEL AND THE INVOLVEMENT OF IMMUNE RESPONSE IN GENETIC RESISTANCE TO MALARIA

Three important aspects highlighted by Williams et al. (1996) have underlying mechanisms that should be considered: 1) α^+-thalassemia status may be linked to an increased proportion of young red blood cells (RBCs), and in fact *P. falciparum* is known to prefer invading young RBCs; 2) the development of an efficient immune response seems to be fundamental in the protection against the disease given by α^+-thalassemia; and 3) nonimmune α^+-thalassemic individuals apparently overcome malarial life-threatening complications and achieve protection from the disease despite an increased incidence of clinical malaria in the first 4 years of age.

Besides offering a general explanation for the genetic resistance to malaria, the Hb/membrane interaction hypothesis seems also to be relevant to α-thalassemia. In fact, unmatched β-globin chains are known to associate with the cytoskeleton and cause oxidative damage to adjacent skeletal proteins due to the globin-associated heme, hemichromes, or iron (Advani et al., 1992).

Figure 1 shows the first point to consider: that the parasite invasion requires the formation of a protein-free patch on the RBC membrane at the invagination site (Destro-Bisol et al., 1996). Integral membrane and cytoskeletal proteins have to be removed from the site of initial contact along the edges of the junction between the parasite and the RBC. The low binding of irreversibly oxidized Hb to the membrane occurring in young RBCs allows a marked rotational mobility of band-3 protein and high membrane deformability, thus facilitating the processes involved in junction formation (Corbett and Golan, 1993).

Bayoumi first proposed in 1987 that HbS might enhance intensity and/or specificity of the host immune response against the *P. falciparum* parasite. According to this author, the immune system in Hb AS subjects

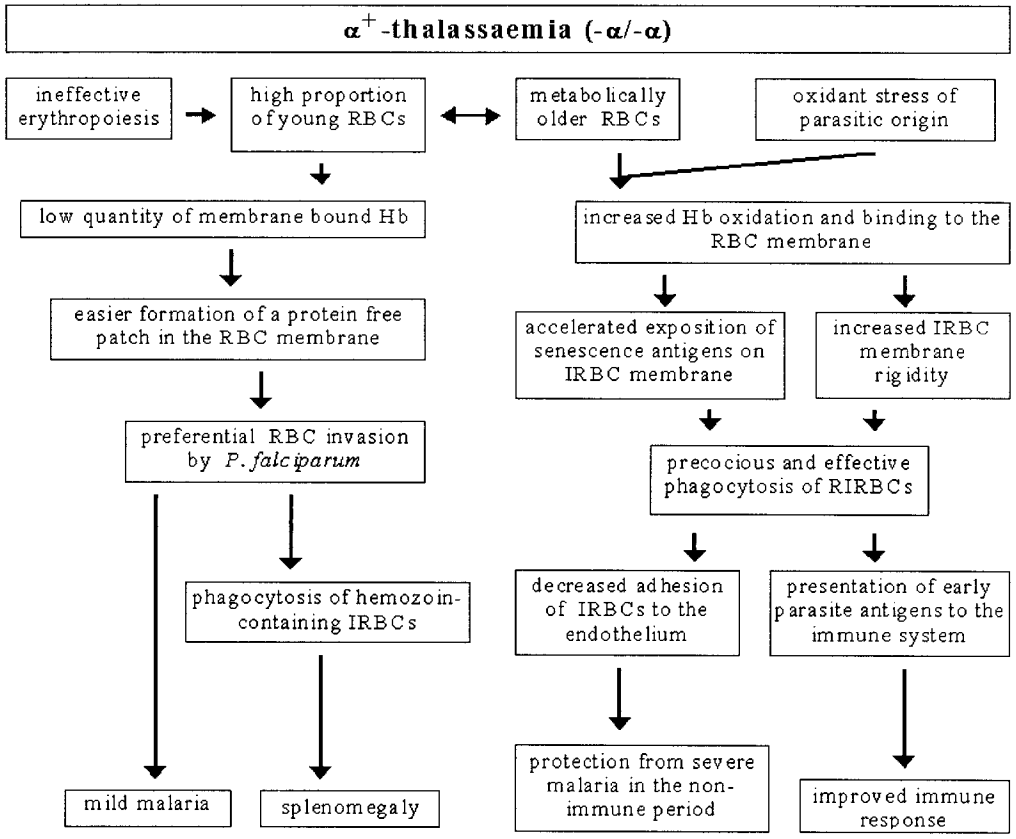

Fig. 1. Schematic summary of the pathways by which the interaction between oxidized hemoglobin and the RBC membrane components may influence the course of *falciparum* malaria in α^+-thalassemic (-α/-α) children.

could interact preferentially with the antigens of the early parasite stages, being less influenced by decoy antigens of mature parasites and other immunosuppressive factors. Exposure of the immune system to early parasite antigens would then drive the acquisition of clinical immunity to malaria in a process which is similar to vaccination. The Hb/membrane interaction may lead to one such early vaccination in α^+-thalassemic children through two distinct pathways. First, the binding of hemichromes to band 3 would trigger a cascade of events leading to the exposure of a senescence antigen on the outer membrane surface, with consequent recognition and removal of infected cells by antigen-driven macrophages. Second, the elastic properties of RBC membranes would be seriously compromised by the formation of peripheral proteins–Hb derivatives complexes, and the anelastic RBCs would be more easily phagocytosed while passing through the spleen slits (see Destro-Bisol et al., 1996). Since the Hb/membrane interaction is considerably enhanced in *P. falciparum*–infected Hb AS and α^+-thalassemic (-α/-α) RBCs, the consequent acceleration of the presentation of the senescence antigen and loss in membrane deformability may facilitate phagocytosis of infected RBCs (IRBCs) already at the ring stage (RIRBCs).

Finally, the Hb/membrane interaction has a protective effect in the nonimmune period as well. In fact, a precocious phagocytosis of RIRBCs will help remove a large amount of circulating IRBCs before the parasites mature and the cells develop knobs on their surface. This may decrease the quantity of

IRBCs which adhere to the venular vascular endothelium of the internal organs and lead to the development of those microcirculatory obstructions which are responsible for most of the deaths from *P. falciparum* malaria (Aikawa et al., 1990). This protective effect may be further enhanced by the fact that the efficiency of phagocytosis is increased when macrophages recognize RBCs which have been infected by the parasite in its early stages. In fact, in such condition there is much less damage to the macrophages caused by hemozoin, a compound that is abundantly produced in the later stages of infected RBCs (Arese et al., 1991). Apart from this specific effect, this optimization of the antimalarial immune activity may also enhance the response to other infectious diseases. In fact, there is a lower incidence of nonmalarial illness in sickle-cell trait carriers and in α^+-thalassemia homozygotes than in normal individuals (Colombo and Felicetti, 1985; Allen et al., 1997).

EVOLUTIONARY IMPLICATIONS OF THE MODEL AND HOW ANTHROPOLOGICAL STUDIES COULD TEST IT

Our reappraisal of the study of Williams et al. (1996) on the susceptibility to malaria in α-thalassemic individuals suggests that the Hb/membrane interaction may be relevant also to those conditions in which the immune response is involved. This further extends the applicability of our model, which was originally designed to explain antimalarial intraerythrocytic modifications (Destro-Bisol et al., 1996). Thus, it appears that the ability to increase the interaction between hemoglobin and the RBC membrane triggers a series of different antimalarial pathways. Two implications arise from this. First, given the importance of the Hb/membrane interaction for normal RBC senescence, it appears that genetic protection against *P. falciparum* is achieved simply by enhancing mechanisms already at work under physiological conditions (Destro-Bisol et al., 1996). This is a parsimonious strategy, as it allows the antimalarial pathways to work within the normal biochemical context of the erythrocyte. Second, there is some malaria resistance which can frequently be found in the same populations. In our perspective, these genes could work together on Hb/membrane interaction to increase resistance against the disease. Therefore, we think a renewed study on the interaction between different genetic factors of resistance to malaria should be of primary importance.

The validation and refinement of our model demands further biochemical evidence. However, anthropological studies are just as necessary to provide a more realistic context as *in vitro* experiments. Two complementary approaches may be pursued. The first one is to analyze the interactions of malaria-resistance genes with genetic polymorphisms which affect the erythrocyte redox status. An example of this is provided by our analysis of the relationships between the polymorphisms at the hemoglobin beta chain (HBβ) and red-cell glutathione peroxidase (GPX1) loci in African populations which had been subjected to endemic *falciparum* malaria (Destro-Bisol et al., in press). The erythrocytes of GPX1*2 heterozygotes have a markedly increased peroxidasic activity, which should shelter them from irreversible oxidation and binding of hemoglobin caused by the oxidant stress exerted by *P. falciparum*. According to our model, the GPX1*2 allele would have an epistatic effect on the Hb AS genotype by lowering its protection against *falciparum* malaria. This prediction seems to be confirmed since a clear trend toward a disassociation between the Hb AS and GPX1 2–1 genotypes was observed, and this finding does not appear to be related either to a general decrease of heterozygosity or to alternative biochemical pathways.

A second line of research would be to study the antimalarial effects of certain natural products introduced as a part of a diet or for traditional antimalarial therapy (Etkin, 1997; Greene, 1997; Jackson, 1991). The potential of this approach can be seen in the study conducted by Jackson (1990) in Liberia on influence of a cyanogen-rich diet on the distribution of the Hbβ*S gene. We have already speculated that a decrease in Hb/membrane interaction caused by high dietary intakes of cyanogens could undermine the biochemical processes at the basis of the selective advantage of HbS and consequently lead the Hbβ*S frequency to progres-

sively decline (Destro-Bisol et al., 1996). Further studies could deal with the antimalarial effect of substances which alter the erythrocyte redox status. In fact, while some natural substances have an oxidative action (e.g. divicine, arteminisin, and ascorbate at low concentrations), others (e.g., vitamin A and E and riboflavin) have the opposite effect. In general, high and regular intakes of oxidant substances would be expected to enhance Hb/membrane interaction, with a consequent antimalarial effect in genetically normal individuals and an increase of protection in carriers of malaria-resistance genes. By contrast, substances with antioxidant properties could interfere with the Hb/membrane interaction and as a result could undermine the mechanisms underlying selective advantage of malaria-resistance genes.

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